presently claimed "allosteric competitive" antibodies. Applicants believe that further clarification of that distinction, and the supporting data found in the specification, will provide a better basis for addressing each of the rejections.

Antibodies against intrinsic factor ("IF") have been previously described that appear to bind to the vitamin B12 ("B12") binding site on IF. Such antibodies directly compete with B12 for binding to IF. In other words, B12 and the "directly competitive" antibodies cannot both be bound to IF at the same time. If the binding site on IF is occupied by the directly competitive antibody it is not available for B12 binding, and vice-versa.

If an IF-antibody complex is formed first, therefore, it must dissociate before the binding site becomes available for B12 binding. The rate of dissociation of the IF-antibody complex, where the antibody bound is a directly competitive antibody, is described by a first-order rate constant that is largely <u>independent</u> of the B12 concentration.

In contrast, the "allosteric competitive" antibody of the invention can bind to IF <u>only</u> in the absence of B12. In turn, however, B12 can bind to IF even when the "allosteric competitive" antibody has already been bound.

The data shown in Figure 2 shows that the dissociation rate of the IF-allosteric antibody complex is actually directly dependent on the concentration of B12 present in the sample. In fact, the rate of the observed dissociation is linearly dependent on the concentration of B12. In other words, the addition of B12 is able to "bump" previously bound antibody from its bound position with IF. In contrast, a <u>directly</u> competitive antibody, if previously bound to IF, would be largely unaffected by the addition of B12. Such a complex would have to dissociate at its spontaneous dissociation rate before binding B12.

Applicants respectfully disagree with the Examiner's interpretation of the data shown on Figure 2. The data in Figure 2 demonstrates the results when IF-allosteric competitive antibody complex is first formed.and B12 is added in varying concentrations. It can be seen

antibody-IF-B12 ternary complex is likely formed which then dissociates with first-order kinetics to yield free antibody and IF-B12 complexes. The binding of antibody and B12 to IF only appears to be competitive because the B12-IF-antibody complex is not stable and cannot be directly detected, e.g., by immunoassay.

The data shown in Figure 2 therefore demonstrates that the concentration of B12 can affect the dissociation rate of a preformed complex of IF and allosteric competitive antibody. In stark in contrast, if a directly competitive antibody and B12 were to bind to the same site of IF, increasing concentrations of B12 would have little or no effect on the dissociation rate of previously bound antibody. The antibody binding site of the IF would become available for B12 binding only after dissociation of the directly competitive antibody-IF complex, and the rate of dissociation would be largely unaffected by the concentration of B12.

The Examiner's concerns with respect to the data presented in Figure 2 may alleviated by a fuller explanation of the BIAcore methodology involved. The BIAcore experiment described in the application measures the optical effects of proteins bound to the surface of a biosensor chip. B12, having a FW of 1355, is itself too small to affect the SPR signal generated in the BIAcore experiment. Since the signal is proportional to the mass of bound protein, B12 does not directly affect that signal, leaving only the antibody and IF as being large enough to be detected.

Accordingly, the concentration of B12 did not change during the course of any particular measurement, even though each such measurement was done at a different concentration of B12. Thus, Figure 2 shows a series of resulting first-order dissociations of IF-antibody complexes at different B12 concentrations.

Thus, Applicants have shown that the antibody of the invention does not bind to the B12 binding site. Applicants have further shown, however, that the antibody binds competitively with B12, in that it will only bind to IF in the absence of B12. By way of brief background, IF is believed to exist in at least two forms in the body, each having

dimer, which is then capable of binding to a specific extracellular receptor. In the absence of B12, the protein appears to exist as a monomer that is not capable of binding to that extracellular receptor. Applicants suspect that the antibody of the invention binds to a site on IF that is exposed only in its monomeric form (i.e., when not bound to B12). Since the antibody of the invention does not bind to the B12 binding site, but only binds to IF in the absence of B12, it follows that the antibody binds to a distinct site, which Applicants refer to as an "allosteric" site.

The term "allosteric" was chosen because somewhat analogous allosteric properties have been observed in the context of enzyme activity. The activity of many enzymes can be controlled by positive or negative effectors that bind at sites distinct from the enzyme's own active site. Such effectors are often referred to as allosteric inhibitors or activators, and are said to bind to allosteric sites on the enzyme protein. Because these allosteric effectors bind at sites other than the enzyme's active site, the enzyme is able to simultaneously bind its substrate at the active site as well as its effector at the allosteric site. Binding of the effector often results in a change in the affinity of the enzyme for its substrate.

Thus, Applicants have discovered and have provided a description of allosteric competitive antibodies to a protein, such as IF. The present description is amply sufficient to allow those skilled in the art to distinguish the allosteric competitive antibodies from directly competitive antibodies previously described.

The Examiner also asserts that the description of how to obtain the allosteric antibodies of the invention beginning on page 13 of the specification merely teaches methods of generating IF antibodies using procedures of a relatively generic nature. The Examiner asserts that since no particular peptide or epitope is set forth that one of ordinary skill would not know how to reproducibly obtain Applicant's preferred embodiments and therefore deposit of the clone is required. Applicants respectfully disagree that deposit is required, although we note that a sample of a preferred hybridoma was deposited at the ATCC on March 29, 1991, as mentioned at page 10 of the specification.

Applicants contend that the method of generating antibodies to IF, as described in the specification, is new in spite of the fact that it employs techniques commonly used to screen

and specifically in Claims 7 and 9 (renumbered as claim 8), Applicants teach that after obtaining hybridomas, free endogenous B12 must first be extracted from the culture supernatant. Thereafter one sample of that extracted antibody-containing supernatant is contacted with IF in the presence of B12, and a second sample of that culture supernatant with IF in the absence of B12. Finally, the method involves isolation of those hybridomas that secrete antibodies capable of binding IF only in the absence of B12.

To Applicants' knowledge this extraction and screening method has not previously been described. Once antibodies that bind IF only in the absence of B12 have been identified, in view of the present teaching those skilled in the art will appreciate the manner in which conventional techniques, e.g., a BIAcore technique, can be used to identify those that are allosteric competitive antibodies.

Accordingly, the rejection under Section 112 is respectfully traversed for the reasons provided above. Both the description and the enablement of the invention in the present specification are sufficient.

Similarly, the rejections under Section 102(b) and 103 are respectfully traversed for the reasons set forth above and previously in parent application Serial No. 682,060. None of the references cited address the concept of allosteric competitive binding, as presently claimed, or suggest that identifying and selecting hybridomas that produce such antibodies is desirable.

With respect to the Section 103 rejection, Galfre et al merely describe general methodology involved in the preparation of antibodies, while Chen et al describe a radioassay that involves the use of "pure IF." Nothing in either reference, considered either alone or in combination, teach or suggest the preparation or use of allosteric competitive antibodies in the manner presently claimed.

Galfre et al merely describes how monoclonal antibodies can be obtained but does not describe in detail how to screen or select for hybridomas that secrete antibodies that bind to a desired antigen competitively with a target ligand.

Nothing in Chen et al teaches or suggests that antibodies to IF are useful or desirable. Chen et al merely describe the use of "pure" IF in a radioassay instead of "crude" IF. They

used in the assay is sufficiently pure to use as an immunogen to obtain competitive monoclonal antibodies to IF. The assay described by Chen et al, is not even an immunoassay.

The rejection under Section 102(b) over Smolka et al is respectfully traversed as well. Smolka et al merely describe how to obtain monoclonal antibodies to IF. They do not describe how to identify and select hybridomas that produce allosteric competitive antibodies of the invention. Contrary to the Examiner's assertion, the allosteric competitive antibodies of the invention do not prevent binding of B12 to IF. Rather, it is the presence of B12 that prevents the binding of the antibodies of the invention to IF. Similarly, Smolka et al do not describe extracting endogenous B12 from the culture supernatant before selecting hybridomas that secrete antibodies to IF. As a result, any allosteric competitive antibodies that may be present would not be selected for nor identified as such.

The Examiner suggests that some of the antibodies described by Smolka et al may inherently contain the allosteric characteristics claimed by Applicants. Applicants respectfully disagree. Smolka et al do not describe a method as used by Applicants to obtain the allosteric competitive antibodies of the invention. Nor does the reference describe or suggest that antibodies that bind to IF only in the absence of B12 can or should be selected for. Thus, a person of skill in the art following the reference's teaching would not "inevitably produce" the claimed subject and thus the reference does not inherently teach the invention.

The rejection under Section 103 over Ellis et al is respectfully traversed as well. The Examiner's reliance on Ellis et al is confusing. On the one hand, the Office Action seems to imply that the allosteric competitive antibodies of the present invention can be used in place of the receptor (IF) in Ellis et al. There is certainly no suggestion in the reference supporting such a substitution, nor is it clear what the purpose or result of such a substitution would be.

On the other hand, the Examiner seems to imply that the allosteric antibody of the present invention can somehow be substituted for the autoantibodies described in Ellis et al. However, the Office Action itself identifies the method in Ellis et al as involving "binding

however, the antibody of the present invention would <u>not</u> block the binding of IF to B12, since B12 would be capable of replacing the antibody of the present invention.

Accordingly, Applicants respectfully request reconsideration of the pending rejection and allowance of all pending at an early date.

Respectfully submitted,

Dated: ____________________

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